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Full Length Research Paper

Application of extracts from the poisonous plant, *Nerium Oleander* L., as a wood preservative

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The antifungal properties of poisonous plant extracts from oleanders (*Nerium oleander* L.) were determined when used as a wood preservative. The extract was prepared from oleanders leaves and flowers in 96% ethyl alcohol. The wood blocks of Turkish oriental beech (*Fagus orientalis* L.*)* and Scots pine (*Pinus sylvestris* L.) were impregnated with the extracts. The abilities of the extract to suppress attack by *Postia placenta* (Fr.) (brown rot) and *Trametes versicolor* (L: Fr.) Quel. (a white-rot) was investigated. Treated blocks were exposed to *P. placenta* and *T. versicolor* attacks for 12 weeks by following the soil-block method. While untreated wood specimens have weight loss ranging between 27.37 and 30.66% for *P. placenta* and 8.64 and 24.06% for *T. versicolor*, the wood treated with the extracts is of weight loss between 5.54 and 10.98% for *P. placenta*, and between 5.02 and 28.25% for *T. versicolor*. The lowest weight loss was found to be for beech wood (5.02%) impregnated with the extract of *oleander* at a concentration level of 0.25% against *T. versicolor.* While the highest weight loss was also on the beech wood (28.25%) treated with the same extract at the level of 0.50% concentration against *T. versicolor.* In conclusion, the extracts could be used as effective wood preservative.

Key words: Poisonous plant extracts, *Nerium oleander* L, Decay fungi, *Postia placenta*, *Trametes versicolor*.

INTRODUCTION

Throughout the course of history wood has remained one of the most important renewable natural resources available to man. However, as a natural organic material wood is degraded by many organisms, principally fungi and insects (Schultz and Nicholas, 2002). Therefore, it is generally treated with a chemical preservative to prevent damage by these aggressive biodeteriogens (Craig et al., 2001).

A large number of preservative compounds have been introduced on to the market but many of them have not gained acceptance because of chemical toxicity, low efficacy, high cost, or corrosiveness (Murphy, 1990). Certain wood preservatives have been banned or limited for some applications such as chromated copper arsenate (CCA) in some European countries, the United States, and Japan (Kartal et al., 2004). Pentachlorophenol (PCP) and many biocides were also prohibited at many European countries long time ago due to their detrimental effect on the natural balance and human health. Therefore, in recent years, Wood Preservation Industry prefers non-chemical based and vegetable based chemicals for wood treatments. Since some natural extractives contain tannin or have toxic effects against biotic agents, they could be preferred for protection of wood or wood based objects against destroying organisms (Schultz and Nicholas, 2000).

Ability of natural plant extracts to protect wood against degrading fungi and insects have been one possible approach for developing new wood preservatives (Sen et al., 2002; Kartal et al., 2004). Chang et al. (1998) reported that α-cadinol obtained from Taiwania heartwood possess high antifungal effectiveness. Konodo and

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Imamura (1986) had also investigated the antifungal compounds in heartwood extracts of *Chamaecyparis obtusa* and they deduced that the main antifungal compounds of *C. obtusa* were cadinane skeletal sesquiterpenoids. Digrak et al. (1999) investigated the antimicrobial activities of extracts of mimosa bark; they reported that the extracts had antibacterial activity. Tang et al. (2007) reported that the ethanolic extracts from the bark of *Acacia confuse* exhibited strong antioxidant activity. Schultz and Nicholas (2002) reported that heartwood extracts might be alternative wood preservatives as they have fungicidal and antioxidant properties. Yang and Clausen (2006) investigated antifungal effect of seven essential oils; derived-ajowan, dill weed, *Egyptian geranium*, lemongrass, rosemary, tea tree, and thyme, and their results indicated that these natural extracts had antibacterial and antifungal activity.

Oleander is one of the most poisonous plants and contains numerous toxic compounds. The most significant of these toxins are oleandrin and neriine, which are cardiac glycosides. They are present in all parts of the plant, but are most concentrated in the sap. Many of Oleander's relatives have similar leaves and contain toxic compounds. It is thought that Oleander may contain many other unknown or un-researched compounds that may have dangerous effects. Oleander bark contains rosagenin, which is known for its strychnine-like effects. The entire plant including the milky white sap is toxic and any part can cause an adverse reaction. Oleander is also known to hold its toxicity even after drying (Inchem, 2005).

The objective of this study was to determine the efficacy of natural poisonous plant extracts from *Oleander* (*N. oleander* L.) in suppressing *P. placenta* and *T. versicolor* attacks to treated sapwood of Turkish oriental beech (*F. orientalis* L.*)* and Scots pine (*P. sylvestris* L.).

MATERIALS AND METHODS

Preparation of extract and wood specimens

The oleanders' leaves and flowers used in this study for decay fungi were collected from the region of Mugla-Turkey in September. The collected samples were air dried and kept in the Herbarium of Muğla University-Turkey.

The oleanders' leaves and flowers were ground into particles with 1 – 2 mm, blended with 100 ml ethyl alcohol then for each trial and placed into the alcohol bath at 50°C for 5 h. The resultant extract solution was filtered through a glass wool filter and then rinsed with a small quantity (about 30 ml) of 96% ethyl alcohol. The extracts solutions were evaporated to constant weight under reduced pressure at 40°C. Subsequently, the extracts were diluted by distilled water and stored in the deep freezer, and later lyophilized in a freeze dryer.

For impregnation process, wood specimens [19 (tangential) x 19 (radial) x 19 (longitudinal) mm] were prepared from air-dried sapwood of Turkish oriental beech *(F. orientalis* L.) and Scots pine (*P. sylvestris* L.).

P. placenta (Fr.), M. Larsen et Lombard (Mad 698), (a brown-rot) and *T. versicolor* (L: Fr.) Quel. (FFPRI 1030: Fungal accession number of Forestry and Forest Products Research Institute, Tsukuba, Japan) (a white-rot) were used as wood-decaying fungi.

Treatment method

Air-dried wood specimens were impregnated in vacuum desiccators with the extracts. Vacuum was applied for 30 min at 760 mm Hg before supplying the solution into the treatment chamber followed by another 30 min at 760 mm Hg diffusion period under vacuum. The carrier solvent used was 100 ml distilled water for each extract concentration such as 0.25, 0.50 and 3.00%.

The total number of treated specimens was 80 in other words, 5 replicate by 4 different concentration levels by 2 different sapwood specimens by 2 fungi. The impregnated wood specimens were weighed (T_2), air dried for one week at ambient temperatures, and then dried in an oven at 60°C for 3 days. Calculation of the amount of preservative absorbed by wood specimen, that is the retention, as kilograms per cubic meter (kg/m^3) were as follows:

$$\text{Retention, kg/m}^3 = (G \times C/V) \times 10 \quad (1)$$

Where $G = (T_2 - T_1)$ = amount in grams of treated solution absorbed by the wood specimen (g), T_1 = initial weight of the conditioned wood specimen before impregnation (g), T_2 = weight of the wood specimen immediately after impregnation and wiping (g), C = grams of preservative in 100 g of treating solution, and V = volume of wood specimens (cm^3).

After the wood specimens were impregnated and weighed to obtain the amount of absorption, they were spaced on trays and exposed to room conditions for 72 h. Afterwards, all such blocks were placed in the conditioning chamber for 21 days to enable them to achieving equilibrium moisture content (EMC). Finally, weights of the specimens were measured (T_3).

Decay test

The treated and untreated wood specimens to be used for decay test were spaced on trays and exposed to room conditions for 72 h. Afterwards, all the wood blocks were placed in the conditioning chamber for 21 days to enable them to achieve equilibrium moisture content (EMC).

Prepared culture bottles filled with 120 cm^3 of soil mixture and 62 g distilled water were added to each bottle. This amount of water to be added was calculated according to "Water Hold Capacity" and "Water required" that clarificated in ASTM D 1413-76. Bottles were sterilized in an autoclave for 30 min at 121°C. The sterilized soil culture bottles were thoroughly cooled. Approximately 10 mm in diameter square fungus inoculums sections was cut from a Petri dish culture, and placed in contact with an edge of the feeder strip on the soil. The soil was then effectively inoculated with the fungal species, and incubated at 27°C and 72.0% relative humidity for 3 weeks. Then, wood specimens were subjected to a modified decay resistance test. Five replicate specimens of each wood type were dried to constant weight and steam-sterilized at 100 ± 2°C for 20 min. After cooling, wood specimens were placed in the culture bottles under air laminar flow condition to avoid contamination. Screwed bottles cap were loosen one-quarter turn, and then exposed to *P. placenta* (Fries) M. Larsen et Lombard (Mad 698), (brown rot) and *T. versicolour* (L.ex Fr.) Quel. (FFPRI 1030) (a white-rot) fungi, in a modified soil-block test according to ASTM D 1413-76 test method for solid wood. For incubation period for 3 months, blocks held at 27°C and a relative humidity (RH) of 75%. At the end of the incubation period, blocks were removed from the test bottles and the mycelium was carefully brushed off the samples. Tested specimens were then reweighed after 4 weeks seasoning to reach relative humidity in the open laboratory. Mass loss was cal-

Table 1. Weight loss of wood samples treated with *Nerium oleander* extract after 12 weeks exposure to fungi.

Wood species	Concentration (%)	Retention level (kg /m^3)	Weight loss (%) due to fungus	
			P. placenta	***T. versicolor***
Turkish oriental beech (*Fagus orientalis* L)	Control	0.00	27.37± 3.36	24.06 ± 3.70
	0.25	0.02	7.55 ± 1.63**	5.02 ± 2.31**
	0.50	0.04	6.33 ± 0.01**	28.25 ± 0.79
	3.00	0.20	5.54 ± 0.96 **	23.24 ± 1.27
Scots pine (*Pinus sylvestris* L)	Control	0.00	30.66 ± 4.00	8.64±1.71
	0.25	0.01	10.00 ± 1.93**	6.63 ± 0.72 **
	0.50	0.02	10.98 ± 1.74**	7.23 ± 1.40
	3.00	0.19	8.76 ± 1.44 **	20.23 ± 2.46

**Significant at 5 % level.
Values are mean ± SD (standard deviation).

culated from the conditioned weight of the wood specimen immediately before and after testing, as follows:

$$\text{Weight Loss (\%)} = (100\ (T_3 - T_4) / T_3) \quad (2)$$

Where T_3 = weight of wood specimen plus remaining preservative after conditioning and before exposure to the test fungi (g), and T_4 = weight of the wood specimen after test and after final conditioning (g).

RESULTS AND DISCUSSION

Percent weight loss caused by two decay fungi after 12 weeks are presented in Table 1. The results reveal that beech wood impregnated by *N. oleander* extracts at 0.25, 0.50 and 3.00% is effective in suppressing attack of *P. placenta,* but 0.50 and 3.00% were not effective against *T. versicolor.* Also for Scots pine, impregnation with the same extracts at 0.25, 0.50 and 3.00% is effective in suppressing the attack of *P. placenta.* On the other hand, concentration of 3.00% was not effective against *T. versicolor* for Scots pine. *N. oleander* extracts at 0.50 and 3.00% may have nutritive properties for *T. versicolor* on the beech wood. This may be attributed to the organic materials such as sugar, protein, etc. in the extracts at the above-mentioned concentration levels. It can that low concentration were more effective than high concentrations against to *T. versicolor*. In a similar research, Goktas et al. (2007) studied the ability of *S. candida* (SB Candidum Mathew) extract to suppress attack by *P. placenta* and *T. versicolor*, and they had obtained similar results.

Oleander is one of the most poisonous plants and contains numerous toxic compounds; the most significant of these toxins are oleandrin and neriine, which are cardiac glycosides (Inchem, 2005). We deduced that, at the low concentrations, the effective substance can easily be dissolved in solutions and affects the fungi. But at high concentrations, the amount of nutritive material increases. This may be attributed to the organic materials such as sugar, protein, etc., in the extracts at high concentrations. Ozen (2005) also observed that *Gynadriris sisyrinchium* (L.) Parl, another poisonous plant, is effective against *P. placenta* and *T. versicolor*. There are large amounts of phenol-glucosidic compounds in the bulbs of the *G. sisyrinchium* (L.) Parl. (Rahmana et al. 2003; Damirov et al., 1996) which inhibits the growth of *P. placenta* on beech wood and Scots pine. It is thought that Oleander may contain many other unknown or unresearched compounds (Inchem, 2005), that may have effects on the fungi. These compounds may prevent wood from the attack of microorganisms.

It may be of concern that application of oleander on interior furniture will portray a risk. However, all of furniture has to be coated. Moreover, the amounts of the poisonous substances used in the study were very little. Even so, oleander is used as drug for miscellaneous pharmaceutical product and other therapeutic preparation. Preparations containing the active principles were formerly used as rodenticides, insecticides, and as remedies for indigestion, fever, ringworm, malaria, leprosy, venereal diseases and as abortifacients. Therapeutic use of oleander glycosides as cardiac drugs were assessed and documented in the 1930s. The USSR pharmacopoeia contains an oleandrin solution (solution Neriolini) and oleandrin tablets. The oleandrin solution contains 22 mg oleandrin, 74 mL alcohol, with distilled water to 100 mL, while oleandrin tablets each contain 100 mg of the active principle (Inchem, 2005).

Conclusion

Decay resistance of wood specimens treated with aqueous solutions of *N. oleander* extract was studied. The wood blocks of Turkish oriental beech (*F. orientalis* L.*)* and Scots pine (*P. sylvestris* L.) were impregnated with poisonous extracts from *N. oleander.* The effects of

the extracts on the developments of *P. placenta* (Fr.) (a brown rot) and *T. versicolor* (L: Fr.) Quel. (a white-rot) were ascertained. The lowest weight loss was found to be for beech wood (5.02%) at a concentration level of 0.25% *N. oleander* extract against *T. versicolor* after 3 months of decay exposure. The highest weight loss was on the beech wood (28.25%) treated with the extract at 0.50% concentration against *T. versicolor.* The most effective dosage of *N. oleander* extract was 0.25%. All concentrations of *N. oleander* were found efficacious in suppressing attack of *P. placenta.* The extract could be considered an effective wood preservative when used against *P. placenta*. Furthermore, the development of more environmentally friendly wood treatments should encourage scientists to exploit plant extracts as wood preservatives since they are characterized by low cost, low mammalian toxicity, and ease of handling and treatment.

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